Substitute Page 1



SPECIFICALLY TARGETED ANTIBODY AGENT, COMPOSITION, KIT & RELATED USES

BACKGROUND OF THE INVENTION

Related Patents

This application is a divisional of US Patent Application Serial No. 08/482,596, filed June 7, 1995, which has been allowed; which in turn is a divisional of USSN 08/162,402 filed December 3, 1990, now US Patent No. 5,972,337; which is a continuation-in-part of USSN 07/607,538, now US Patent No. 5,455,031.

Technical Field

invention relates to a polypeptide having This kDalton **HMFG** 46 specificity of the antibody binding polynucleotide, and а differentiation antigen, a polyribonucleotide encoding it, anti-polypeptide antibodies, methods of detecting the polypeptide and DNA and RNA encoding it, a method of imaging cells expressing the polypeptide, a method of detecting the presence of the polypeptide in a biological fluid by binding the antibody to the polypeptide, in vivo and ex vivo methods of delivering a therapeutic agent to a target cell expressing the polypeptide, a fusion protein of the polypeptide and at least one other polypeptide, labeled polynucleotides and polyribonucleotides encoding the polypeptide and a complementary DNA sequence, method of detecting RNA and DNA by hybridization with labeled probes, a method of vaccination with the polypeptide, and method of treating breast cancer with an anti-sense DNA.

WHAT IS SEEKED TO BE PATENTED AS NOVEL & UNOBVIOUS IN LETTERS PATENT OF THE UNITED STATES IS:

- 52. (Canceled)
- 53. (Canceled)
- 54. (Canceled)
- 55. (Canceled)
- 56. (Canceled)
- 57. (Canceled)
- 58. (Canceled)
- 59. (Canceled)
- 60. (Canceled)
- 61. (Canceled)
- 62. (Canceled)
- 63. (Canceled)
- 64. (Canceled)
- 65. (Canceled)
- 66. (Canceled)
- 81. (Amended) An in vivo method of imaging a cancer of epithelial origin or cells expressing a polypeptide having the antibody binding specificity of the about 46 Kd differentiation Human Milk Fat Globule (HMFG) antigen, comprising

administering to a subject suspected of being afflicted with the cancer or carrying the cells an amount of a detectably labeled or unlabeled specifically targeted antibody, comprising a monoclonal antibody selectively binding a 46 Kd MW HMFG differentiation antigen that has an antigen affinity constant about 10^{10} - 10^{5} M⁻¹, and an agent comprising a detectable label, the antibody and the agent being operatively linked to one another, under conditions effective to deliver the antibody to target cells of epithelial origin carrying at least a portion of the 46Kd MW HMFG differentiation antigen in the subject's body to form antibody-cell antigen complexes;

administering to the subject a detectably labeled agent that binds the antibody at a site other than the 46 kDalton HMFG polypeptide binding site if the antibody is unlabeled; and detecting the presence of a label in the subject's body.

- 82. (Amended) The method of claim 81, wherein the antibody is administered intravenously, intraperitoneally, intracavitarily, intra-tumor, intramuscularly, or into the lymphatic system.
- 83. (Amended) The method of claim 81, wherein the labeled agent comprises a fuorescent or radiolabeled agent.
 - 84. (Amended) The method of claim 81, wherein

the antibody comprises an unlabeled antibody; and

the labeled agent comprises a labeled anti-antibody immunoglobulin, antibody binding fragment thereof, protein A, or Protein C.

85. (Amended) The method of claim 81, further comprising upon label detection the delivery of a therapeutic agent to target cancerous cells or cells of epithelial origin by

binding a therapeutic agent to the antibody of claim 81, at a site other than its antigen binding site;

administering to the subject a therapeutically effective amount of the antibody-bound therapeutic agent under conditions effective for the antibody to deliver the agent to the target cells; and

allowing the antibody to bind to the target cells, and the therapeutic agent to exert its effect on the cells.

- 86. (Canceled)
- 87. (Canceled)
- 88. (New) The method of claim 81, wherein the labeled agent comprises a radionucleide, a fluorescent label, an enzyme or biotin.
- 89. (New) The method of claim 81, wherein the labeled agent is detected as a conjugate.

- 90. (New) The method of claim 89, wherein the antibody is conjugated to avidin, streptavidin, or a magnetic bead.
- 91. (New) The method of claim 81, wherein the antibody comprises a monoclonal antibody.
- 92. (New) The method of claim 81, wherein the antibody is provided as a composition with a non-proteolytic carrier.
- 93. (New) The method of claim 92, wherein the carrier comprises a biologically acceptable carrier.
- 94. (New) The method of claim 93, wherein the carrier comprises a pharmaceutically acceptable carrier.
- 95. (New) The method of claim 85, wherein the therapeutic agent comprises a radionucleide, an immmunotoxin, or an enzyme.
- 96. (New) The method of claim 85, wherein the antibody-therapeutic agent is delivered as a conjugate.
- 97. (New) The method of claim 96, wherein the antibody-therapeutic agent is conjugated to avidin, streptavidin, or a magnetic bead.
- 98. (New) The method of claim 85, wherein the antibody-therapeutic agent comprises a monoclonal antibody.
- 99. (New) The method of claim 85, wherein antibody-therapeutic agent is provided as a composition with a non proteolytic carrier.
- 100. (New) The method of claim 99, wherein the antibody-therapeutic agent carrier comprises a biologically acceptable carrier.
- 101. (New) The method of claim 100, wherein the antibody-therapeutic agent carrier comprises a pharmaceutically acceptable carrier.

Table 1:

DNA Sequence and Derived Amino Acid Sequence of BA46-1 cDNA.

	*		0	*		20 		*	30 I		*	4	1	*		50 		
GAT Asp	TTC Phe	ATC Ile	CAT His	GAT Asp	GTT Val	AÀT Asn	AAA Lys	AAA Lys	CAC His	AAG Lys	GAG Glu	TTT Phe	GTG Val	GGT Gly	AAC Asn	TGG Trp	AAC Asn	
*	60)	*		70	*		80		*	90		*	1	.00	*	•	
AAA Lys	AAC Asn	GCG Ala	GTG Val	CAT His	GTC Val	AAC Asn	CTG Leu	TTT Phe	GAG Glu	ACC Thr	CCT Pro	GTG Val	GAG Glu	GCT Ala	ĊAG Gln	TAC Tyr	GTG Val	
110 i		*	120		*			*			*				*	160		
ÁGA	TTG Leu	TAC Tyr	CCC Pro	ACG Thr	AGC Ser	TGĊ Cys	CAC His	ACG Yhr	GCC Ala	TGC Cys	ACT Thr	CTG Leu	ĊGC Arg	TTT Phe	GAG Glu	CTÀ Leu	CTG Leu	
	*	170		*	180)	*	1	.90	*	r	200		*	210)	*	
GGC Gly	TGT Cys	GAG Glu	CTG Leu	AAC Asn	GGA Gly	TGC Cys	GCC Ala	AAT Asn	CCC Pro	CTG Leu	GGC Gly	CTG Leu	AAG Lys	AAT Asn	AAC Asn	AGC Ser	ATC Ile	
_	220		•	230		*					250					*	270	
CCT Pro	GAC Asp	AAG Lys	CAG Gln	ATC Ile	ACG Thr	GCC Ala	TCC Ser	AGC Ser	AGC Ser	TAC Tyr	AAG Lys	ACC Thr	TGG Trp	GGC Gly	TTG Leu	CAT His	CTC Leu	
	*	2	280	,	:			*	300		*	3	10	+	+	320		
TTC Phe	AGC Ser	TGG Trp	AAC Asn	CCC Pro	TCC Ser	TAT Tyr	GCA Ala	CGG Arg	CTG Leu	GAC Asp	AAG Lys	CAG Gln	GGC Gly	AAC Asn	TTC Phe	AAC Asn	GCC Ala	
*	330)	*	:	340	,	+	350 1		*	360 1)	*	3	370 I	+	k	
TGG Trp	GTT Val	GCG Ala	GGG Gly	AGC Ser	TAC Tyr	GGT Gly	AAC Asn	GAT Asp	CAG Gln	TGG Trp	CTG Leu	CAG Gln	GTG Val	GAC Asp	ĊTG Leu	GGC Gly	TCC Ser	
380		*	390		*	4 (00	*	4	110		*	420		*	4	430	
TCG Ser	AAG Lys	GAG Glu	GTG Val	ACA Thr	GGC Gly	ATC Ile	ATC Ile	ACC Thr	CAG Gln	GGG Gly	GCC Ala	CGT Arg	AAC Asn	TTT Phe	GGC Gly	TCT Ser	GTC Val	
*	440		*	450)	*	4	160	*		470 		*	480)	*		
CAG Gln	TTT Phe	GTG Val	GCA Ala	TCC Ser	TAC Tyr	AAG Lys	GTT Val	ĠCC Ala	TAC Tyr	AGT Ser	AAT	GAC Asp	AGT Ser	GCĠ Ala	AAC Asn	TGG Trp	ACT Thr	
490		*	500	*		510	0	*	!	520	,		530		*	540	0	
ĠAG Glu	TAC Tyr	CAG Gln	GAC Asp	CCC Pro	AGG Arg	ACT Thr	GGC Gly	AGC Ser	AGT Ser	AAG Lys	ATC Ile	TTC Phe	CĊT Pro	GGC Gly	AAC Asn	TGG Trp	GAC Asp	
		!	550		*	560		*	57	0	*		580		* !	590		
AAC Asn	CAC His	TCC Ser	CAC His	AAG	AAG	AAC	TTG									1		
*				гуу	Lys	Asn	Leu	Phe	GAĠ Glu	ACG Thr	CCC Pro	ATC Ile	CTG Leu	GCT Ala	CGC Arg	TAT Tyr	GTG Val	
	600		*	61	Lys	Asn *	Leu	Phe	GAĠ Glu	Thr	CCC Pro 630	ATC Ile	стG Leu *	GCT Ala	Arg	TAT Tyr	GTG Val	
CGC Arg	600 ATC Ile	CTG Leu	* CCT	61 I GTA	Lys O GCC	Asn * TGG	Leu	Phe 620 AAC	Glu	Thr * ATC	Pro 630 I GCC	Ile	teu * CGC	Ala 64 CTG	Arg 0 GAG	Tyr * CTG	Val	
CGC Arg 650	ATC Ile	CTG Leu *	* CCT	61 I GTA	Lys O GCC	Asn * TGG	CAC His	Phe 620 AAC	Glu CGC Arg	Thr * ATC	Pro 630 I GCC	Ile	teu * CGC	Ala 64 CTG	Arg 0 GAG	Tyr * CTG	Val CTG Leu	
Arg 650 GGC	ATC Ile	teu *	* CCT Pro 660 I	61 GTA Val	Lys GCC Ala * CCT	* TGG Trp 67 GCC	CAC His	Phe 620 AAC Asn	Glu CGC Arg	* ATC Ile 680	Pro 630 GCC Ala	CTG Leu	* CGC Arg 690	64 CTG Leu	Arg 0 GAG Glu *	* CTG Leu 70	CTG Leu	703
Arg 650 GGC Gly TGC GGG	TGT Cys	* TAG End TTG GGT	* CCT Pro 660 TGG (GCT	61 GTA Val CCA SEQ.	GCC Ala * CCT NO: CAG AGG	Asn * TGG Trp 67 GCC 2) CCC CAG	CAC His ACC	Phe 620 AAC Asn CCC TAA CAC	CGC Arg AGG ATC CAC	Thr * ATC Ile 680 TCT ACC ACA	Pro 630 GCC Ala TCC ATA GTC	CTG Leu * TGC	* CGC Arg 690 TTT CTG CCT	Ala 64 CTG Leu CCA	Arg GAG Glu * TGG ACT TCC	* CTG Leu 700 GCC GGG CTC	CTG Leu 0 CGC GAA TTT	757 811
Arg 650 GGC Gly TGC GGG CCC	TGT Cys CTC GAG ACC	* TAG End TTG GGT CTC	* CCT Pro 660 TGG (GCT GTT CAC	61 GTA Val CCA SEQ. TCT CAG CTC ACT	Lys GCC Ala * CCT NO: CAG AGG TCA GTC	Asn * TGG Trp 67 GCC 2) CCC CAG CGG CTG	CAC His * ACC CTT CAC GCC TTT	Phe 620 AAC Asn CCC TAA CAC CTG TCT	CGC Arg AGG ATC CAC CCC TAG	* ATC Ile 680 TCT ACC ACA CAG GCA	Pro 630 GCC Ala TCC ATA GTC CCC CTG	TGC GGG ACC CTA AGG	* CGC Arg 690 ITTT CTG CCT AGC GAT	CTG Leu CCA GGG CCC CCC	Arg I GAG Glu * TGG ACT TCC GTC AGT	* CTG Leu 700 GCC GGG CTC CCC AGG	CTG Leu CGC GAA TTT TAA TCT	757 811 865 919
Arg 650 GGO Gly TGO GGO CCO GGO ACO	TGT Cys CTC GAG CCA GAG GAG GCC	TAG End TTG GGT CTC GTC GAC GAT	* CCT Pro 660 TGG (GCT GTT CAC CTC AGG CCC	61 GTA Val CCA SEQ. TCT CAG CTC ACT AAA AGG	Lys GCC Ala * CCT NO: CAG AGG TCA GTCA GTCT GGGG TGC	* TGG Trp 67 GCC 2) CCC CAG CGG CTGG CTAA GTG	CAC His * ACC CTT CAC GCT TTGT	Phe 620 AAC Asn CCC TAA CAC CTCT AGG CTC	CGC Arg AGG ATC CAC CAC TAG GCG TGT	* ATC Ile 680 TCT ACC ACA CAG GCA TGT CTC	Pro 630 GCC Ala TCC ATA GTC CCC GGT TCC	TGC GGG ACC CTA AGG TTC TAG	* CGC Arg 690 TTT CTG CCT AGCT CCT CCC	CTG Leu CCA GGG CCC CTG GCC CTC	Arg I GAG Glu * TGG ACT TCC GTCT ACT TCT	* CTG Leu 700 GCC CCC AGG GTC CAC	CTG Leu CGC GAA TTT TAA TCGG ACA	757 811 865 919 973 1027
Arg 650 GGG GGC CCC CCC GGG ACC TCA	TGT Cys CTC GAG CCA CCA CCA CCA CCA CCA CCA CCA CCA	teu * TAG End TTG GGT CTC GAC GAC GAT TCC CTT	* CCT Pro 660 TGG TGGTT CAC CTC AGG CCC CAT GCC	61 GTA Val CCA SEQ. TCT CAG CTC ACT AAA AGG GGT	Lys GCC Ala * CCT NO: CAG AGG TCA GTC GGG TGC GGG CGG	Asn * TGG Trp 67 GCC 2) CCC CAG CGG CTG CAA GTC CCC	CAC His ACC CTT CAC GCC TTT AGT TGT AAG	Phe 620 AAC ASN CCC TAA CAC CTG TCT AGG CTC AAA GTC	CGC Arg AGG ATC CAC CCC TAG GCG GGC GGC	* ATC Ile 680 TCT ACC ACA CAG GCA TGT CTCG CCT	Pro 630 GCC Ala TCC ATA GTC CCC CTG GGT TCC GAA GGG	TGC GGG ACC CTA AGG TTC TAGG CCTA	* CGC Arg 690 TTT CTG CCT AGC CCA CCA	GGG CCC CTG GCC TGT	Arg I GAG Glu * TGG ACT TCC AGT CCT TCGT TGG GCC	* CTG Leu 70 GCC CCC AGG GTC CAGA ACA	CTG Leu 0 CGC GAA TTA TCT CGG ACA TTAA	757 811 865 919 973 1027 1081 1135
Arg 650 GGG GTy TGG GGG CCC CCC GGG ACC TCA TGA GGT TGA	TGT Cys CTC CATG CCATG CATG	TAG End TTG GGT CTC GAC GAT TCC GAT TCC GTTG GGG	* CCT Pro 660 TGG CTCAC CAC CAC CAC CAT GCC TCAC CAC CAC CAT	GTA Val CCA SEQ. TCT CAG CTC ACT AAA AGG GGT TGT TGA	Lys GCC Ala * CCT NO: CAG AGG TCA GTC GGC CGG CCC GGC CGG CCC GGC GGC CGG GTC GGC GG	Asn * TGG Trp 67 GCC CAG CGG CTG CGG CTG CTC CCAA AGA	CAC His * ACC CTT CAC GCC TTT AGT AAG TGC GCC GCC GCC GCC GCC GCC GCC GCC GC	Phe 620 AAC ASN CCC TAA CAC CTG TCT AGG CTC AAA GTC ACA ACA ACA ACG ACT ACT ACG ACT	CGC Arg AGG ATC CAC CCC TAG GCG GGC TCC CAT CCC CAT CAC CCC TCC GGC CAT CCC	* ATC Ile 680 TCT ACA CAG GCA TGTC CCG CCT ACA GGG	Pro 630 GCC Ala TCC ATA GTC CCC CTG GGT TCC GAA GGGG TTGT	TGC GGG ACC CTA AGG TTC GCC GTA CTC GCG GCA	* CGC Arg 690 TTT CCG GAT CCCA CCA CCGA GGG	CCA GGGGCCCCCCTGGCTGGTGGTGGTGG	Arg I GAG GAG GIU * TGG ACT TCC AGT CCT TGG GCC TGC GTC GTC	* CTG Leu 70 GCC CCC AGG GTC CAGA ACA CTAT TTT	CTG Leu 0 CGC GAA TTT TAA TCT CGG ACA TTAA ACT TCT GGG GGG	757 811 865 919 973 1027 1081
Arg 650 GGG GTy TGG GGG ACG TGG GGT TGG GGG GGG GGG GGG GGG GGG G	TGT Cys CTC CATG CCATG CATG	* TAG End TTG GGT CTC GAC GAT TCC GAT GCG GAT GCG GAT GCG GAT GCG GAT GCG GAT GCG GAT	* CCT Pro 660 I TGG TGGTT CAC CTC AGG CCC TCC AGG CCC CCC AGG CCC CCC AGG CCC CCC AGG CCC CCC	GTA Val CCA SEQ. TCT CAG CTC ACT AAA AGGT CGT TGT TGAG GCT	Lys GCC Ala * CCT NO: CAG AGG TCA GTC GGG CGG CGG CGG CTTT TTT	TGG TTP 67 67 CCC CAG CTG CTG CTG CTAA CAA CAA	CAC His * ACC CTT CAC GCC TTT TGT AGT TGC GAC GCG GCG GCG GCC	Phe 620 AAC Asn CCC TAA CAC CTG TCT AGG CTC ACA GTC ACA GGC AGA	CGC Arg AGG ATC CAC CCC TAG GCG GCG TCCC CAG GGC CAT GGA	* ATC Ile 680 TCT ACC ACAG GCA TGT CTC CCT AAG GCA GCA GCA GCA GCA GCA GCA GCA GCA	Pro 630 GCC Ala TCC ATA GTC CCTG GGT TCC GAGG TGT GGG TGT GGG AGG	TGC GGG ACC CTA AGG TTC GCC GTA CTC GCG GCA	* CGC Arg 690 TTT CCG GAT CCCA CCA CCGA GGG	CCA GGGGCCCCCCTGGCTGGTGGTGGTGG	Arg I GAG Glu * TGG ACT TCC GTCC TGC TGC GCC TGC GTCG AAA	* CTG Leu 70 GCC CCC AGG GTC CAC AGA ACA TTAT TTAT	CTG Leu CGC GAA TTTAA TCT CGG ACA TTAA ACT TCT TGGGG	757 811 865 919 973 1027 1081 1135 1189

Potential n-linked glycosylation sites are underlined.

<u>Table 2</u>: Comparison of Derived BA46-1 Amino Acid Sequence with C-terminal Human Serum Factors V and VIII

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46 Kda F I H D V N K K H K E F V G N W N K N A V H V N L FAV F K G N S T R N V M Y F N G N S D A S T I K E N Q FAVIII Y R G N S T R N V M Y F F G N V D S S G I K H N I

F E T P V E A Q Y V R L Y P T S C H T A C T L R F E L L G K F D P P I V A R Y I R I S P T R A Y N R P T L R L E L G K F N P P I I A R Y I R L H P T H Y S I R S T L R M E L M G

C E L N G C A N P L G M E N G K I E N K Q I T A S S S F K K C D L N S C S M P L G M E S K A I S D A Q I T A S S F K K C D L N S C S M P L G M E S K A I S D A Q I T A S S Y F T

T W G L H L F S W N P S Y A R L D K Q G N F N A W V A G S S W W G D Y - - W E P F R A R L N A Q G R V N A W Q A K A N M F A T - - - W S P S K A R L H L Q G R S N A W R P Q V Y Y G N D Q W L Q V D L G S S K E V T G I I T Q G A R N F G S N N N N K Q W L E I D L L K I K K I T A I I T Q G C K S L S N N N P K E W L Q V D L G S S K E V T G I I T Q G C K S L L S N N N P K E W L Q V D L G S S K E V T G V T T Q G V K S L L S S E M Y V K S Y T I H Y S S Q Q G V E W K P Y R L K S S M V T E M Y V K E F L I S S S Q D G H Q W T L F F Q N - - G K S E M Y V K S Y T I H Y S S Q Q G V E W K P Y R L K S S M V T E M Y V K E F L I S S S Q D G H Q W T L F E T P I L A R Y V V R I D K I F E G N T N T K G H V K N F F N P P I I I S R F I R V V K V F Q G N Q D S F T P V V V N S L D P P L L T R Y L R I L P V A W H N R I A L R L E L L G C (SEQ. No. 3) I P K T W N Q S I A L R L E L L F G C D - - - I Y (SEQ. No. 5)
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An arrow indicates Junction of C1 and C2 repeats.